PERSPECTIVES: IMMUNOLOGY

Memory T Cells—Local Heroes in the Struggle for Immunity

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Immunity to pathogens relies on the ability of the immune system to "remember" past infections. This property, known as immunological memory, has been recognized for several decades and the success of vaccination regimens depends on it. Yet, the immune cells and molecular machinery responsible for maintaining immunological memory have remained surprisingly elusive. Two articles, one from Masopust and colleagues on page 2413 of this issue (1) and the other from Reinhardt et al. in a recent issue of Nature (2), identify some of the essential features of immune memory. In particular, the investigators uncover a dramatic redistribution of immune cells from lymphoid tissues to nonlymphoid tissues following an immune response, which explains how immunity and memory are maintained throughout the body.

It is now clear that lymphoid tissues, such as the lymph nodes and spleen, are the sites where a primary immune response develops (see the figure). Lymph nodes are designed to collect antigens from the tissues they "drain," and to accommodate massive influxes of T and B lymphocytes from blood through specialized microvessels called high endothelial venules (see the figure). They also provide the appropriate backdrop for interactions between T and B cells and antigen-presenting cells. Rapid antigen recognition depends on the highly efficient movement of very rare antigen-specific T and B cells through the lymphoid tissues. Naïve T cells are known to recirculate throughout the body by shuttling between the blood and lymphoid tissues, and evidence suggests that they are incapable of entering nonlymphoid tissues (3). However, those naïve T cells that respond to antigen in lymphoid tissues undergo profound changes in the surface molecules that they express and in their activities. Some differentiate into effector cells that kill invading pathogens or stimulate B cells to make antibody against for-

eign antigens; others become memory cells that have the capacity to differentiate into effector cells when they reencounter antigen. Effector and memory T cells provide a lasting systemic immunity by migrating to nonlymphoid tissues, particularly sites such as the skin or mucosal membranes where pathogens are first encountered.

The Masopust and Reinhardt papers now provide a fascinating illustration of the critical transformation that T lymphocytes undergo: from naïve T cells that home to lymphoid tissues, to fully-fledged effector or memory T cells that migrate to all areas of the body. Masopust et al. (1) tracked the migration of CD8$^+$ memory T cells with tetramers composed of major histocompatibility complex (MHC) molecules bound to an antigenic peptide. Their tetramer was composed of mouse MHC class I molecules and a peptide derived from vesicular stomatitis virus (VSV). This tetramer identified VSV-specific CD8$^+$ T cells in mice that had been infected with this virus. The dynamics and distribution of VSV-specific CD8$^+$ T cells
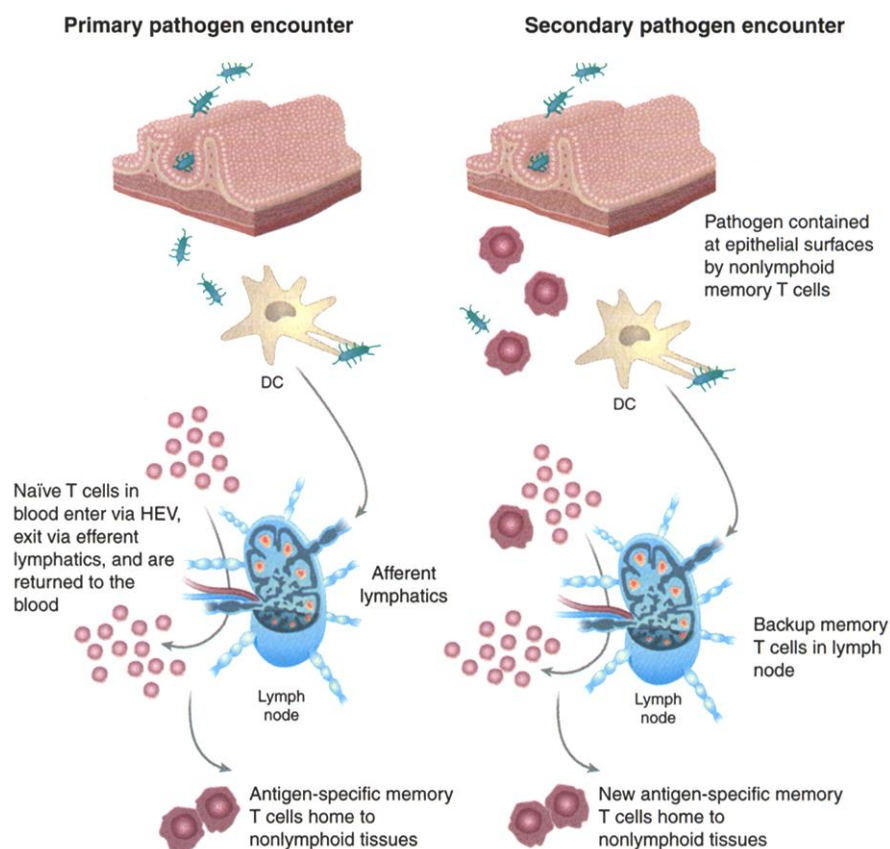
Well-traveled T cells. (Left) First encounter with a pathogen. Dendritic cells (DCs) scour tissues throughout the body for antigens including pathogens, and then transport them to local lymphoid tissues (such as the lymph nodes) through afferent lymphatic ducts. The high endothelial venules (HEV) facilitate the continuous and rapid entry of naïve T cells from blood into lymphoid tissues. Antigen recognition results in clonal expansion of antigen-reactive T cells and their differentiation into memory T cells, which acquire new surface adhesion molecules and chemokine receptors. Memory T cells leave the lymph nodes through the efferent lymphatics and migrate throughout the lymphatic and circulatory systems. **(Right)** A repeat encounter with a pathogen. Rapid memory T cell responses are generated in nonlymphoid tissues, such as the skin or lamina propria of the gut, when pathogens are re-encountered. After restimulation with antigen, these nonlymphoid memory T cells rapidly become efficient effector lymphocytes. Should they become overwhelmed by pathogen, then backup is provided by the memory T cells in lymphoid tissues. Lymphoid memory T cells are not mobilized as rapidly as nonlymphoid memory cells, but they do recirculate between blood and lymphoid tissues. During the first few months after pathogen challenge, it is primarily the abundant nonlymphoid memory T cells that provide immunity.

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were revealed by analyzing which T cells bound to the tetramer. Remarkably, 9 days after infection, nonlymphoid tissues including kidney, liver, and peritoneum contained extremely high numbers of VSV-specific CD8$^+$ T cells (which constituted up to 40% of the total CD8$^+$ population). Even after 296 days, some tissues still retained VSV-specific T memory cells that constituted as much as 4% of the total CD8$^+$ population. Despite the high proportion of VSV-specific CD8$^+$ cells in nonlymphoid tissues, this T cell subpopulation had almost totally disappeared from lymphoid tissues. Thus, the clonally expanded effector and memory T cells had become redistributed to the body's nonlymphoid tissues, the very places where protection against pathogens is needed the most (see the figure on the previous page).

Reinhardt et al. (2) reached a similar conclusion although they tracked the redistribution of CD4$^+$ T cells using a totally different approach. They followed migrating antigen-specific CD4$^+$ T cells by transferring naïve T cells with a defined antigenic specificity (derived from a T cell receptor transgenic mouse) into recipient mice and using Thy-1 as a marker for the transferred cells. The authors painstakingly determined the presence of antigen-reactive CD4$^+$ T cells in all tissues of the recipient animals. They did this by immunohistochemical analysis of whole-body sections using an antibody against Thy-1.1 that only bound to donor-derived T cells. With this protocol, they first tracked the migration of transferred naïve T cells, and then monitored changes in their migration after injection of the specific antigen. As expected, naïve T cells initially became localized only within lymphoid tissues. However, after injection of antigen and a primary immune response, there was a striking redistribution of antigen-reactive T cells to nonlymphoid tissues including the liver, lungs, and intestinal lamina propria. The redistribution pattern was very similar to that described by Masopust et al. for CD8$^+$ T cells. These two reports, together with findings in sheep (3), mice (4), and humans (5), provide definitive evidence of the redistribution of effector and memory T cells to nonlymphoid tissues after exposure to antigen.

As T lymphocytes differentiate from naïve cells to effector and memory cells, they acquire new surface molecules (for example, ICOS or different CD45 isoforms) that are involved in their interactions with other cells or in signal transduction events. There are also changes in the expression of surface proteins that facilitate cell movement, such as the adhesion molecules L-selectin, CD44, and various integrins, as well as numerous chemokine receptors, particularly CCR5, CXCR3, and CCR7 (3–5). Altered expression of these surface molecules reflects the new journeys that effector and memory T cells must take. The chemokine receptor CCR7 is a useful marker because its expression indicates a capacity for homing to lymphoid tissues: All naïve T cells express CCR7, whereas many antigen-stimulated T cells do not. Recently, Sallusto et al. (6) distinguished two types of antigen-activated T cells—a CCR7-negative "effector-memory" T cell and a CCR7-positive "central-memory" T cell. After restimulation with antigen, CCR7-negative effector-memory T cells rapidly execute effector activities such as cytokine secretion or cytotoxicity, whereas CCR7-positive memory T cells take longer to become mobilized.

What both Masopust et al. and Reinhardt et al. demonstrate is that the T cells redistributing to nonlymphoid tissues, which are deployed rapidly at the initial sites of pathogen entry, have an activation profile characteristic of Sallusto's effector-memory T cells. In contrast, lymphoid memory T cells, which are slower to become effector cells, form a reserve that can be called upon when the frontline memory cells are overwhelmed. But, there is one notable difference between the Masopust and Reinhardt results. In the former study, lymphoid memory T cells were rare, whereas in the latter, they constituted 50% of the entire long-lived memory T cell population. These diverging outcomes could be due to differences between CD4$^+$ and CD8$^+$ memory T cell populations, or may reflect the nature of the antigen used (live virus versus recombinant peptide).

A controversy that has never been fully resolved is the contribution of antigen to the maintenance of memory. One of the implications of the Masopust work is that immunological memory after pathogen challenge can be divided into a short-term phase, lasting weeks to months, followed by a long-term phase, lasting many years (at least for some pathogens). During the short-term phase, the proportion of antigen-specific effector or memory T cells in some tissues is as high as 20 to 40% of the total T cell population. Given the enormous number of different pathogens that the body encounters, obviously such a large response against one pathogen could not be sustained. But this short-term "intensive" memory probably serves the individual well during the period immediately after pathogen invasion when the infectious burden is greatest. After this initial burst, long-term memory then becomes established. A classic example of long-term immunological memory is found among the Faroe Islanders, who, 65 years after a measles epidemic, still retained immunity to the virus. Is it possible that short-term memory depends on the survival and activity of nonlymphoid memory T cells, whereas long-term memory depends on lymphoid memory T cells? The answer is perhaps, although some nonlymphoid memory T cells persist for at least 296 days, regardless of whether they are restimulated with antigen or not (1). Persistent restimulation by retained, reencountered, or cross-reactive antigen may promote short-term memory; long-term memory is known to be maintained independently of antigen (7).

What are the wider implications of the two studies? First, T cell migration is indeed a highly rational process, and a clear distinction can be made between the migration pathways of naïve T cells and those of tissue memory T cells. Second, some new features of immunological memory have been revealed. Rapid memory responses occur because antigen-reactive cells are greatly expanded in number and have redistributed to numerous tissues to provide "frontline" immune protection. But there is more to immunological memory than simply an increase in the number of antigen-reactive cells. Both studies demonstrate that memory T cells migrating to nonlymphoid tissues can rapidly become effector cells. The unleashing of functional subsets of T cells into nonlymphoid tissues is obviously important for immune protection, but there may be a downside as well. New environments might trigger autoimmune reactions as a result of T cell cross-reactivity with self antigens that have not been encountered before. The importance of nonlymphoid memory T cells for long-term immunological memory of different pathogens remains to be elucidated. Regardless of the outcome, immunologists need to ponder the fact that a subset of memory T cells, situated in nonlymphoid tissues and rarely sampled, may in fact be the true heroes in the struggle for immunity.

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